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Assessment of lead toxicity to *Desulfovibrio desulfuricans* G20: influence of components of Lactate C medium

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Abstract

The bioavailability and toxicity of lead (Pb) to *Desulfovibrio desulfuricans* G20 is greatly influenced by aqueous phase chemical composition. Apparent Pb toxicity is reduced by precipitation and complexation with chemicals found in standard growth media for sulfate-reducing bacteria (SRB). To determine the influence of medium composition on observed Pb toxicity, a new medium was developed to more accurately assess the toxicity of Pb to *Desulfovibrio desulfuricans*. The new medium, metal toxicity medium (MTM), eliminates abiotic Pb precipitation and minimizes formation of Pb complexes in solution. Significant growth of *Desulfovibrio desulfuricans* was observed on MTM in the absence of Pb, while no measurable growth was observed at 3 mg/l Pb as $PbCl_2$. For comparison, in Lactate C medium (Burlage et al., 1998) abiotic Pb precipitation was apparent, and the specific growth rate at 100 mg/l Pb was only reduced by 8.1% compared to the Pb-free control. Toxicity was measured in terms of longer lag times and slower growth rates (including no growth) as compared to Pb-free controls. This report describes the effects of specific medium components on Pb toxicity to *Desulfovibrio desulfuricans* and provides a better baseline for comparison of natural and industrial waters for observing heavy metal toxicity on SRB. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Bioavailability; *Desulfovibrio desulfuricans*; Heavy metal; Lead; Medium; Sulfate-reducing bacteria; Toxicity; Zinc

1. Introduction

Sulfate-reducing bacteria (SRB) play an important role in precipitation of heavy metals in natural waters and some wastewaters (White et al., 1998). Under anaerobic conditions, SRB utilize the sulfate ion as an electron acceptor during the oxidation of organic material, forming hydrogen sulfide that forms insoluble complexes with many heavy metals (Gadd and Griffiths, 1978; Mueller and Steiner, 1992; Rittle et al., 1995; Poulson et al., 1997). The remediation or treatment of waters containing heavy metals and/or radionuclides in various digesters, reactors, or in natural waters is based on the metal precipitating reactions mediated by SRB (Jackson, 1978; Barnes et al., 1991; Clancy et al., 1992; Hammack and Edenborn, 1992; Pado et al., 1994; Kafkewitz et al., 1994; Abdelouas et

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al., 1999; Cassinis et al., 1999; El-Bayoumy et al., 1999; Lloyd et al., 1999; Shokes and Moller, 1999). However, for efficient treatment of waters containing heavy metals by SRB either in situ or ex situ, there must be sufficient knowledge of the toxicity of various heavy metals to SRB populations. Bacterial sulfate reduction will not take place if heavy metals are present at concentrations that are toxic to the target organisms. The toxicity of heavy metals to sulfate-reducing bacteria has been described (Booth and Mercer, 1963; Saleh, et al., 1964; Temple and LeRoux, 1964; Capone et al., 1983; Loka Bharathi, et al., 1990; Hao et al., 1994; Song et al., 1998; Jalali and Baldwin, 2000). However, little attention has been focused on the influence of chemical species present in the toxicity assay medium.

In this study, important factors necessary to quantify the bioavailability and toxicity of Pb to *Desulfovibrio desulfuricans* were systematically addressed. These factors include abiotic precipitation and complexation of Pb due to reaction with the following medium components found in Lactate C medium (Burlage et al., 1998); phosphate, sulfate, yeast extract, reductants (ascorbic acid and sodium thiogycollate), and metal chelator (sodium citrate). The Lactate C medium was modified by reducing concentrations of some medium constituents and eliminating non-essential medium components. An alternative medium, called metal toxicity medium (MTM), was developed that minimized the effects of abiotic precipitation and complexation of Pb, while still providing a medium suitable for growth of *Desulfovibrio desulfuricans*. We realize that in many, if not most, natural and industrial settings, there will be precipitating and complexing agents present. These agents will not, however, always be the same as those present in Lactate C medium. MTM may provide somewhat of a baseline for comparison of natural and industrial waters. In addition, we envisage that in future studies, MTM could be supplemented with specific precipitating and complexing agents to help better understand the interactive effects of toxic heavy metals and other components in the aqueous system. In addition to medium development, techniques were developed and tested to eliminate the precipitation of Pb sulfide due to the reaction with sulfide initially present in the inoculum. Carry-over of even trace quantities of sulfide can reduce the bioavailability of heavy metals and hence, significantly influence the observed metal toxicity.

2. Materials and methods

2.1. Chemicals

The medium components were purchased from Fisher Scientific (Pittsburgh, PA, USA) with the exception of the following. Yeast extract and tryptone were obtained from the Difco Chemical Company. PIPES buffer (1,4-Piperazinediethane sulfonic acid, disodium salt monohydrate) resazurin and sodium sulfate were obtained from the Aldrich Chemical Company, USA. All chemicals used were of analytical grade.

2.2. Microorganism and cultivation conditions

Desulfovibrio desulfuricans G20 was maintained in Lactate C medium (Burlage et al., 1998). As part of a larger project to study the interactions of SRB, mineral surfaces, and heavy metal contaminants, *Desulfovibrio desulfuricans* G20 was modified to contain the GFP plasmid (Neal et al., in press) to allow direct observation of fluorescence on mineral surfaces. To maintain the GFP plasmid, 20-μg/ml chloramphenicol was added to the medium prior to inoculation. Initial Pb toxicity work was started with Lactate C medium, but it soon became apparent that this medium formulation produced significant artifacts in toxicity studies. In addition to the medium formulation, modifications to typical medium inoculation procedures were necessary to prevent the precipitation of Pb-sulfide due to reaction with sulfide initially present in the inoculum. Hydrogen sulfide initially present in an active inoculum was removed by flushing with ultra-pure nitrogen for one hour.

To examine Pb bioavailability and subsequent toxicity, *Desulfovibrio desulfuricans* was grown with and without Pb in Lactate C medium and in subsequently modified media. One hundred milliliters of autoclave-sterilized medium and a 5-ml inoculum (36–48 h culture broths previously sparged with ultra-pure nitrogen for 1 h) were aseptically transferred into 150-ml serum bottles. Before inoculation of the serum bottles, the inoculum was checked by epi-fluorescence microscope, with an excitation filter of 480 nm and an emission filter of 520 nm. The cells were found motile and fluorescent. All glassware was previously washed with 2 N HNO_3. The inoculated medium was then purged of oxygen by bubbling with ultra-pure nitrogen gas for 4–6 min. The serum bottles were sealed with butyl rubber septa, capped and crimped with aluminum seals, and pressurized with nitrogen at 10 psi. Stock solutions of $PbCl_2$ and $ZnCl_2$ were prepared and added as needed to the serum bottles by syringe. The *Desulfovibrio desulfuricans* was incubated in the serum bottles at 30°C on a shaker operated at 125 rpm. A 1-ml sample was removed from each serum bottle with a 1-ml syringe and analyzed for total cell protein to quantify temporal changes in the concentration of *Desulfovibrio desulfuricans*.

To examine the effect of redox potential (E_h) on the growth of *Desulfovibrio desulfuricans* and Pb toxicity,

resazurin was used as a redox indicator. Resazurin is colorless at 0.5 mg/l in a medium at pH 7 that has a value of $E_h \leq -100$ mV (Twigg, 1945). Resazurin was added to the medium prior to autoclaving. To run the sulfate and lactate-free controls, washed cells of *Desulfovibrio desulfuricans* were used. The cells were centrifuged in the presence of ultra-pure nitrogen gas at $10\,000 \times g$ for 10 min. The supernatant was discarded and the cell pellets were suspended in 0.89% NaCl. This process was repeated twice. Each experiment was carried out in duplicate and repeated twice for each set of conditions. The initial cell protein concentration, as calculated by measuring the inoculum protein concentration and adjusting for dilution, was 1–2 mg/l. The detection limit of the Coomassie assay method was ≈ 5 mg/l of protein. In all experiments, the measured values were plotted.

2.3. Analytical methods

Total cell protein (live and dead biomass) was determined using a quantitative colorimetric Coomassie assay method (Pierce, Rockford, IL). For total cell protein estimation, 0.5-ml samples were taken aseptically from the serum bottles and placed in culture tubes where 0.5 ml of 1 N NaOH was added. Each tube was covered with a steel cap and incubated in an oven at 99°C for 10 min. The culture tubes were left for 15 min to cool at room temperature (24°C). After 15 min, 0.1 ml of 6 N HCl was added to each tube and vortexed. Coomassie reagent (1 ml) was added to each tube, vortexed, and left for 15 min. The absorbance of each solution was measured at 595 nm and compared to a standard curve generated for bovine serum albumin. Samples for aqueous Pb concentration were prepared by filtering through a 0.2-μm membrane filter and diluting with 3% HNO_3. Concentrations of Pb were measured on an Agilent 4500 inductively coupled plasma mass spectrometer (ICP-MS). Sulfide concentration was determined spectrophotometrically using the methylene blue method applied to liquid samples, ranging from 50 μl to 0.5 ml, which had been collected in 0.5 ml of a 10% (w/v in water) zinc acetate solution (Fogo and Popowsky, 1949; Florin 1991).

3. Results and discussion

3.1. Assessment of Pb toxicity to Desulfovibrio desulfuricans G20 in different media

In this study, the toxicity was measured as the prevention or inhibition of growth of *Desulfovibrio desulfuricans* G20 as measured by total cell protein. To accurately assess the toxicity of Pb, it is important to eliminate and/or minimize medium components responsible for abiotic precipitation and solution-phase complexation of Pb. Prior research on microbial metal toxicity has largely overlooked abiotic precipitation and complexation. This significantly influences the critical concentration where metal-induced inhibition is observed (Booth and Mercer, 1963; Saleh, et al., 1964; Temple and LeRoux, 1964; Capone et al., 1983 Loka Bharathi et al., 1990; Hao et al., 1994; Song et al., 1998; Jalali and Baldwin, 2000). In this study, tests were run to determine the toxicity of Pb in Lactate C medium. Lead was added to give a final concentration of 25, 50 or 100 mg/l. Visual inspection of un-inoculated Lactate C medium indicated that Pb had precipitated immediately. A Pb-free control was also included. The growth of the *Desulfovibrio desulfuricans* in Lactate C medium with different concentrations of Pb is shown in Fig. 1. It is evident from Fig. 1 that up to 100 mg/l Pb, the highest Pb concentration tested, the bacteria grew with no significant inhibition of growth rates. With Pb precipitates visible, the bioavailability of the Pb was significantly diminished in this medium and thus, the toxicity of Pb to *Desulfovibrio desulfuricans* was likely to be greatly underestimated.

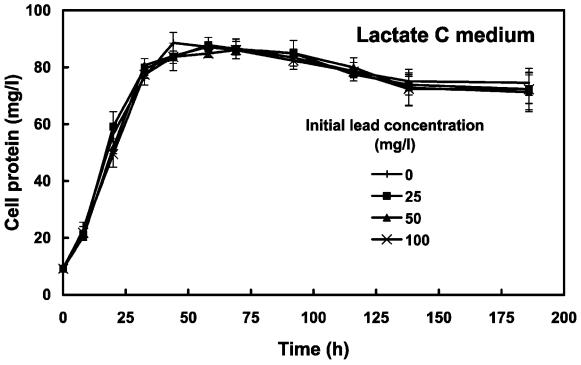

Fig. 1. The growth of *Desulfovibrio desulfuricans* G20 with different concentrations of lead in Lactate C medium.

Individual Lactate C medium components (Table 1) were added to nanopure water and amended to give a final Pb concentration of 100 mg/l. After 3 h, the concentration of Pb in the aqueous phase was measured to determine the contribution of each component to abiotic Pb precipitation. For the individual medium components, Fig. 2 shows that the percentage of Pb that had precipitated from the initial 100 mg/l in the presence of 4.5 g/l sodium sulfate, 2 g/l magnesium sulfate, 6.8 g/l sodium lactate, 1 g/l yeast extract, 0.5 g/l potassium phosphate monobasic and 0.1 g/l each reductant, ascorbic acid and thioglycollate was found to be 88, 73, 8, 20, 96 and 6%, respectively. Fig. 2 clearly shows that in Lactate C medium, any of the components could be responsible for precipitating Pb from solution, thus, decreasing the bioavailability of

Table 1
Media compositions used in toxicity studies of *Desulfovibrio desulfuricans* G20. Unless noted, all values are given in g/l

Constituent	Lactate C medium	Medium A	Medium B	Metal toxicity medium
Sodium lactate	6.8	5.1	5.1	5.1
Sodium sulfate	4.5	2.13	2.13	2.13
Calcium chloride dehydrated	0.06	0.06	0.06	0.06
Sodium citrate	0.3	0.3	0.3	–
Ammonium chloride	1	1	1	1
Magnesium sulfate	2	1	1	1
Potassium phosphate monobasic	0.5	–	–	–
Yeast extract	1	0.1	0.1	0.05
Ascorbic acid	0.1	0.1	–	–
Sodium thioglycollate	0.1	0.1	–	–
Chloramphenicol	20 μg/ml	20 μg/ml	20 μg/ml	20 μg/ml
Tryptone	–	–	–	0.5
PIPES	–	10.93	10.93	10.93

Pb. This decrease in bioavailability is probably due to the formation of Pb phosphate, Pb sulfate and undetermined Pb complexes with lactate, yeast extract and reductants (Babich and Stotzky, 1979). When combined with the data given in Fig. 1, these results indicate that no Pb toxicity was observed because Pb was not bioavailable.

It can be seen that Lactate C medium has a strong potential to mask Pb toxicity effects. Therefore, three different media were developed by significantly modifying the Lactate C medium. Firstly, phosphate buffer was replaced with PIPES buffer (1,4-Piperazine-diethane sulfonic acid, disodium salt monohydrate; 30 mM, pH 7.2), and the concentrations of sodium lactate, sodium sulfate, magnesium sulfate and yeast extract were reduced. These changes yielded medium A in Table 1. However, in this medium, Pb again precipitated after 24 h by forming unknown complexes with reductants and/or yeast extract. To prevent Pb precipitation, reductants were removed from medium A to give medium B (Table 1). In medium B, however, again it was observed that in the presence of 0.1 g/l yeast extract, the Pb precipitated after 3 days by forming unknown complexes with yeast extract components. The concentration of yeast extract was then reduced to 0.05 g/l, but a significant decrease in growth was observed (data not shown). Tryptone at 0.5 g/l was added to the medium to restore the growth of *Desul-*

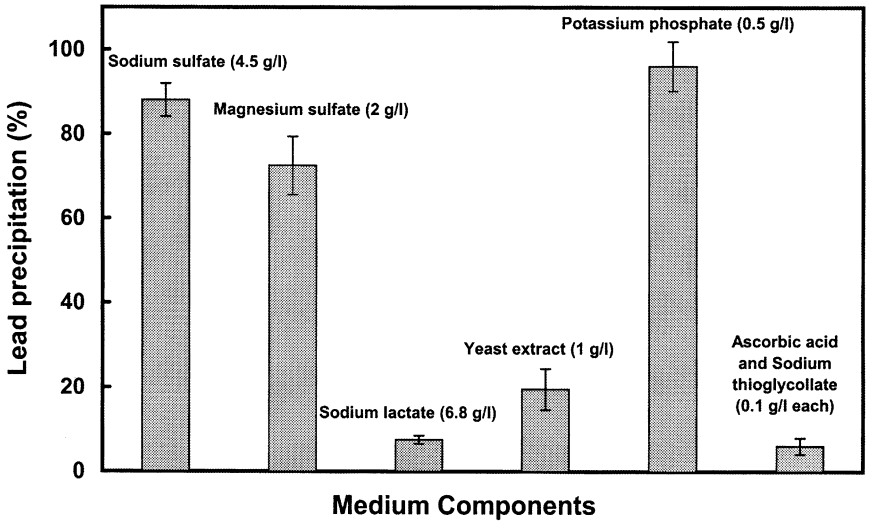

Fig. 2. Chemical (abiotic) precipitation of lead (100 mg/l) by different medium components.

fovibrio desulfuricans. Finally, since sodium citrate can act as a metal chelator (Konishi et al., 1996), it was also removed. Removing the sodium citrate had no significant effect on the growth of *Desulfovibrio desulfuricans* (data not shown). These final changes resulted in a new medium, called metal toxicity medium (MTM) (Table 1) that was used for further toxicity experiments. In MTM, significant growth of *Desulfovibrio desulfuricans* was observed in the absence of Pb. Moreover, no abiotic precipitation of Pb was observed at 25 mg/l Pb, even after a prolonged incubation of four months.

3.2. Growth of Desulfovibrio desulfuricans G20 with lead

The growth of the *Desulfovibrio desulfuricans* with different concentrations of Pb in medium A, medium B and MTM is shown in Fig. 3a,b,c, respectively. In medium A, with 5 and 10 mg/l Pb, lag times of 26 ± 8.5 h were observed (Table 2). In addition to increased lag times, *Desulfovibrio desulfuricans* growth rates decreased with an increase in initial Pb concentration (Fig. 3a). In medium A, there was no reduction in specific growth rate with 3 mg/l Pb, while with 5 and 10 mg/l Pb, a 61.6 and 76.1% respective reduction in specific growth rate was observed (Table 2). It can be seen that by simply changing the pH buffer, the apparent toxicity of Pb is much higher in comparison to Lactate C medium where only a small decrease in specific growth rate was observed for up to 100 mg/l Pb. The reduction in specific growth rate in Lactate C medium at 25, 50 and 100 mg/l Pb was only 2, 2 and 8.1%, respectively. In medium B, the absence of reductants showed no significant effect on the growth of *Desulfovibrio desulfuricans*, with the exception of a slight increase in lag time (Fig. 3b). However, the absence of reductants in the medium had a very noticeable effect on the observed toxicity of Pb to *Desulfovibrio desulfuricans*. No measurable growth was observed with 5 mg/l Pb for up to 250 h. At 0.5 and 1 mg/l Pb, no significant reduction in specific growth rate was observed; however, the lag times were increased to more than 26 ± 8.5 and 75 ± 24 h, respectively (Table 2).

The growth of *Desulfovibrio desulfuricans* in MTM with different concentrations of Pb is shown in Fig. 3c. In MTM with no Pb, there was no significant increase in lag time as compared to medium B (Fig. 3c). With 0.5 and 1 mg/l Pb in MTM, no significant reduction in specific growth rate was observed; however, the lag times were increased, to more than 32 ± 17 and 89 ± 26.9 h, respectively (Table 2). It was also observed that with the treatments containing Pb, even after a long lag phase, once the growth of *Desulfovibrio desulfuricans* started, it attained a total cell protein equivalent to

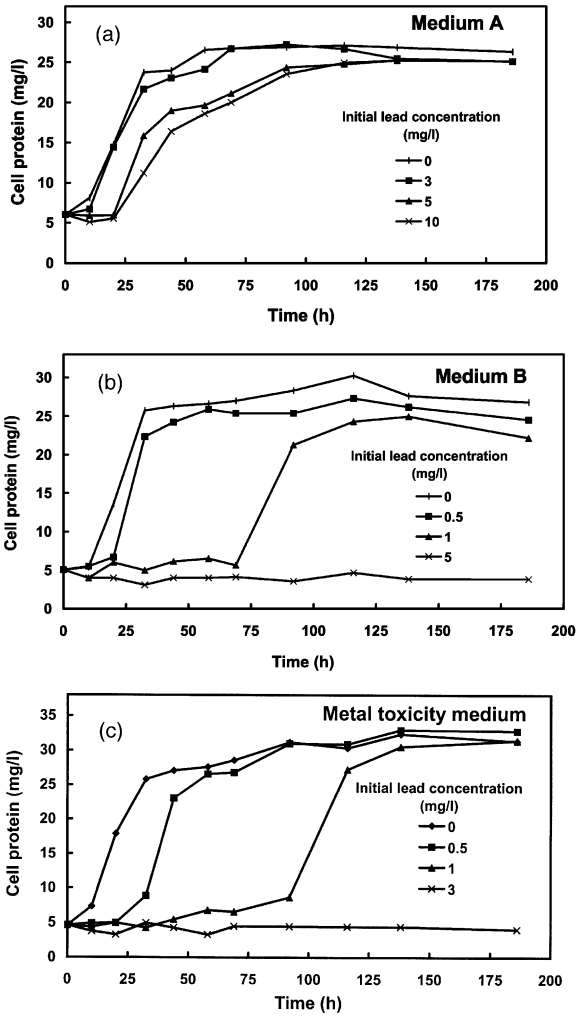

Fig. 3. The growth of *Desulfovibrio desulfuricans* G20 with different concentrations of lead in different media: (a) medium A; (b) medium B and (c) metal toxicity medium.

that of the Pb-free control. No growth was observed in MTM at 3 mg/l Pb even after prolonged incubation (up to 250 h). In addition, at higher Pb concentrations, long lag phases were observed with a maximum lag phase observed to be 108 h. In all the serum bottles, the pH of the medium remained approximately 7.2 throughout the experiments.

From these observations, it can be seen that the choice of pH buffer, the presence or absence of reductants, and the source of micronutrients in microbiological media can have a profound effect on the toxicity of Pb to *Desulfovibrio desulfuricans*, e.g. growth in 100 mg Pb/l in Lactate C as compared to no growth in 3 mg Pb/l in MTM. Similarly, it is expected that in natural systems and wastewater treatment processes, the toxicity of Pb and other heavy metals would heavily depend

Table 2
Influence of medium components on the length of the lag phase and the specific growth rate of *Desulfovibrio desulfuricans* G20 in the presence of Pb

Medium	Pb concentration (mg/l)	Lag time, t_L (h)[a]	Maximum specific growth rate, μ^b (h^{-1})
Lactate C medium	0	–[c]	0.1485 ± 0.0006
	25	–	0.1455 ± 0.0043
	50	–	0.1454 ± 0.0221
	100	–	0.1364 ± 0.0027
Medium A	0	14 ± 8.5	0.1283 ± 0.0317
	3	14 ± 8.5	0.1323 ± 0.0361
	5	26 ± 8.5	0.0493 ± 0.0530
	10	26 ± 8.5	0.0306 ± 0.0253
Medium B	0	15 ± 7.1	0.1032 ± 0.0095
	0.5	26 ± 8.5	0.0914 ± 0.0231
	1	75 ± 24.0	0.0601 ± 0.0165
	5	NG[d]	NG[d]
Metal toxicity medium	0	14 ± 8.5	0.1186 ± 0.0368
	0.5	32 ± 17.0	0.1286 ± 0.0222
	1	89 ± 26.9	0.0998 ± 0.0414
	3	NG[d]	NG[d]

[a] Values are reported as average ± standard deviation.
[b] Specific growth rate (μ) = $dx/(x dt)$.
[c] Not checked.
[d] No growth.

on local water characteristics. It is envisaged that MTM could be used as somewhat of a baseline for comparison of natural and industrial systems, with amendments added as necessary to better reflect the actual complexing and precipitating agents found locally.

3.3. Redox potential (E_h) in MTM during growth of *Desulfovibrio desulfuricans* G20

One of the concerns with the MTM formulation was that without the reductants, the E_h would be too high for growth and survival of *Desulfovibrio desulfuricans* G20. The initial E_h of MTM was very high and the medium was blue in color in the presence of 0.5-mg/l resazurin. To determine whether the increase in lag times and the decrease in specific growth rates were due to toxicity, and not a E_h artifact, *Desulfovibrio desulfuricans* were grown with different concentrations of Pb and zinc (Zn) in the presence of resazurin. It was observed that with no Pb present, inoculated controls with reductants (ascorbic acid and sodium thioglycolate at 0.1 g/l each) became colorless after 6 h of incubation, while in the absence of reductants, the serum bottles became colorless after 9 h. Resazurin at 0.5 mg/l at a circum-neutral pH, becomes colorless at an $E_h \leq -100$ mV (Twigg, 1945). At 9 h, the remaining serum bottles that contained either Pb or zinc were pink in color, but after 12–14 h, all the serum bottles were colorless indicating that the E_h in all bottles was ≤ -100 mV. Therefore, for *Desulfovibrio desulfuricans,* reductants are not necessary for growth; however, the lack of reductants can increase the lag phase somewhat.

To ensure that the drop in E_h was not due to a carry-over of sulfide with the inoculum, the cells were washed in the presence of ultra-pure nitrogen gas by centrifugation at $10\,000 \times g$ for 10 min. The supernatant was discarded and the cell pellets were suspended in 0.89% NaCl. This process was repeated twice. The washed cells of *Desulfovibrio desulfuricans* were homogenized in 10% zinc acetate and analyzed for sulfide content. No sulfide could be detected. In further controls, washed cells of *Desulfovibrio desulfuricans* were inoculated into sulfate-free and lactate-free media in the presence of resazurin. All these inoculated controls became colorless after 14–16 h of incubation. Even though the medium was colorless, there was no measurable depletion of sulfate in the lactate-free control. No sulfide could be detected in either the sulfate- or the lactate-free control. At the time of this submission, un-inoculated controls have remained blue for more than a month. In summary, these observations indicate that the decrease in E_h was not the result of sulfide carry-over in the inoculum. In addition, in the sulfate-free controls, the decrease in E_h cannot be attributed to depletion of sulfate and concomitant sulfide production. These controls clearly show that *Desulfovibrio desulfuricans* can lower the E_h in MTM

and the addition of reductants is not necessary. Finally, in addition to visual observations, cell growth in MTM was quantified by measuring the protein concentration of the culture. As shown in Fig. 4, after 36 h, there was no significant difference in growth in two controls (no Pb or Zn, with or without reductants). Both controls showed good growth of *Desulfovibrio desulfuricans*. However, in treatments that contained Pb, no detectable growth was observed with 1 or 5 mg/l Pb after 36 h. These results indicate that the toxicity effect is not an E_h artifact of the medium, since Pb exhibited a distinct toxicity to *Desulfovibrio desulfuricans* at 1 mg/l, but no toxicity was observed at 1 mg/l Zn.

It was hypothesized that the inhibition of *Desulfovibrio desulfuricans* by Pb was the result of Pb-sulfide complexes, thus, preventing sulfide from reducing the E_h of the medium. *Desulfovibrio desulfuricans* was subjected to two parallel treatments using Zn, rather than Pb, at 1 and 5 mg/l. In contrast to the treatments containing Pb, the two Zn treatments showed good cell growth. As compared to the Zn- or Pb-free controls, at 1 and 5 mg/l Zn, a 7.8 and 49.2% inhibition in growth was observed, respectively. A comparison of the Fig. 4 data for 1 mg/l Zn and 1 mg/l Pb is of particular interest. At pH 7, the solubility of zinc sulfide in pure water is 2.3×10^{-7} mg/l and the solubility of Pb sulfide is 5.8×10^{-10} mg/l (Devegt et al., 1998), so that both Pb and Zn would complex with sulfide to remove nearly all metal from solution. However, with 1 mg/l zinc, no significant toxic inhibition was observed, whereas in the parallel test with 1 mg/l Pb, no measurable growth was observed. It is clear from these results that Pb exhibits a distinct toxicity to *Desulfovibrio desulfuricans* even at 1 mg/l, and that the toxicity effect is not an E_h artifact of the medium or the presence of a sulfide scavenger.

4. Summary and conclusions

In natural and man-made systems, sulfate-reducing bacteria contribute significantly to metal immobilization by precipitation as a sulfide complex. For efficient treatment of waters containing heavy metals by SRB either in situ or ex situ, there should be sufficient knowledge of the toxicity of various heavy metals to SRB populations. Bacterial sulfate reduction will not take place if heavy metals are present at levels that are toxic to the target organisms. Numerous reports are available that discuss the toxicity of heavy metals to SRB, but many of these reports are very qualitative in nature due to their experimental design. Most prior research on metal toxicity to SRB has largely overlooked the effects of abiotic precipitation and complexation that significantly influences the critical concentration where metal-induced inhibition is observed. In this study, it is shown that the choice of pH buffer, the presence or absence of reducing agents and metal chelators can have a profound effect on the observed bioavailability and toxicity of Pb to *Desulfovibrio desulfuricans*. As a result, Lactate C medium has been modified to minimize the reaction of medium components with Pb in order to retain Pb in a bioavailable state that shows toxicity to *Desulfovibrio desulfuricans*. In MTM, growth was inhibited at 0.5 mg/l Pb and no growth was observed at 3 mg/l (up to 250 h). In Lactate C medium, due to medium components, Pb shows little toxicity as measured by cell growth, even at 100 mg/l Pb. It is evident from these results that Pb exhibits a distinct toxicity to *Desulfovibrio desulfuricans* in MTM even at 0.5 mg/l and that the toxicity effect is not an E_h artifact of the medium.

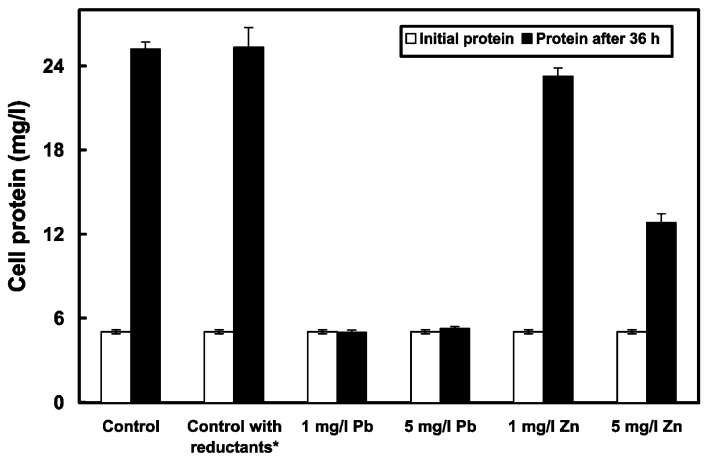

Fig. 4. Effects of reductants*, Pb and Zn on the growth of *Desulfovibrio desulfuricans* G20. *Ascorbic acid and sodium thioglycollate, 0.1 g/l each.

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